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Appendix D

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Thesis title: THE MOLECULAR GENETIC BASIS OF NATURAL VARIATION IN TRICHOME DENSITY IN ARABIDOPSIS THALIANA

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**The molecular genetic basis of natural variation in trichome  
density in *Arabidopsis thaliana***

**A thesis presented in partial fulfilment of the requirements**

**for the degree of**

**Doctor of Philosophy**

**in**

**Plant Biology**

Institute of Fundamental Sciences

Massey University

Palmerston North

New Zealand

Rebecca Bloomer

2013



## ABSTRACT

Understanding the genetic basis of natural variation in phenotypes is a central, yet often elusive, goal in evolutionary biology. Trichome density, an herbivory defence trait in *Arabidopsis thaliana*, is a powerful model for investigating natural phenotypic variation, combining a genetically well characterised developmental pathway with a quantitatively and qualitatively variable phenotype of selective importance. Here, Quantitative Trait Locus (QTL) mapping and candidate gene analyses were undertaken to explore the genetic basis of variation in trichome density in natural accessions of *A. thaliana*, under an overarching hypothesis that allelic variation in the epidermal development pathway plays a significant role.

QTL mapping for constitutive and wounding-induced trichome density and for plasticity of density was undertaken in two newly developed mapping populations, broadening the range of allelic variation sampled in trichome density studies. This study is the first to address the genetic architecture of induced density and plasticity, finding a genetic basis to plasticity and a surprising negative response to wounding among some members of the populations used. Some QTL mapped are unique, while others appear common to both constitutive and induced density phenotypes or to overlap across mapping populations, suggesting particular loci may be key to generating variation for trichome phenotypes. Epistatic interactions and candidate genes for QTL within, up- and downstream of the epidermal development pathway are identified.

Candidate gene analyses focussed on genes within the epidermal development pathway: the trichome-specific MYB *GL1* and the pleiotropic WD-repeat *TTG1*. In both *GL1* and *TTG1*, a pattern of high frequency polymorphism correlates with variation in trichome density. In *GL1*, variation has both qualitative and quantitative effects, with both protein-coding and regulatory changes proposed as underlying bases. The *TTG1* coding region is subject to strong purifying selection, and the observed quantitative effect on density appears to be based on variation in regulatory sequence. Both QTL mapping and candidate gene approaches support the hypothesis of a key role for the epidermal development pathway in generating variation in trichome density in *A. thaliana*, and more generally a role for variation in regulatory genes contributing to natural phenotypic variation.



## ACKNOWLEDGEMENTS

In researching and writing this thesis I have had the support of a fantastic group of people, without whom it may not have been started, finished, or half the fun. Many thanks first to my advisor, Dr Vaughan Symonds. I could probably count on one hand the number of times it really was “just a quick question”, and I truly appreciate your time and expertise. Thanks to both you and Dr Jen Tate for fostering a supportive and challenging lab to grow up in - it is a pleasure to be a part of a lab with such great people and diverse research interests.

A number of people provided specific assistance along the way. Thanks to Professor Alan Lloyd for kindly donating *ttg1* and *gl1* mutant lines and transgenics vectors, and Jay Jayaraman and Dr Xiao Song for donating *Agrobacterium* stocks and helping me to get transgenics up and running in our lab. Thanks also to the many others who have lent reagents and lab equipment, given helpful advice and asked thought-provoking questions. My studies were supported by a Tertiary Education Commission Top Achievers’ Doctoral Scholarship, and I am grateful also for further travel support from both the New Zealand Society of Plant Biologists and the Institute of Fundamental Sciences.

The PhD experience would have been a lonely one without my LoST Lab (& Associates) colleagues, past and present: Todd, Matt, Rowan, Tina, Cindy, Fronny, Nick, Jill, Megan, Kay, Jessie, Amir and Prashant. Thank you all for your moral support, science and life advice, and ability to find the funny side in almost anything. Thanks also to my friends in the wider world, particularly Ruth, Rechelle and Emma, for your support, and for reminding me to get out once in a while.

I am also deeply grateful to my families, the Page/Bloomers, Ridens and Pursers, who have both modelled and inspired a love of learning and been unfailingly supportive of my long career as a student. I am especially thankful to Dan for planting the idea very early on and to Helen and Vicky for their encouragement when I most needed it. Lastly and mostly, I am thankful for the love, encouragement and patience of my husband, Dean - it’s been an adventure, and I am glad I have you to share it with.



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## ABBREVIATIONS

$\mu\text{M}$	Micromolar (micromoles per litre)
$\mu\text{mol}$	Micromole
$\mu\text{L}$	Microlitre
ABRC	<i>Arabidopsis</i> Biological Resource Centre
AI-RIL	Advanced Intercross-Recombinant Inbred Line
ANOVA	Analysis of variance
bHLH	Basic Helix-Loop-Helix
bp	Base pair
CASS	Cheaply amplified size standard
cDNA	Complementary deoxyribonucleic acid
cm	Centimetre
cM	CentiMorgan
CTAB	Hexadecyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
GWAS	Genome-wide association study
$H^2$	Broad-sense heritability
HO	Hi-0 x Ob-0
HOC	Hi-0 x Ob-0 Constitutive
HOD	Hi-0 x Ob-0 Damaged
INRA	Institut National de la Recherche Agronomique/French National Institute for Agricultural Research
kb	Kilobase pairs
LB	Luria-Bertani (agar or broth)
LD	Linkage disequilibrium
LOD	Logarithm of odds
MAGIC	Multi-parent Advanced Generation InterCross
mg	Milligram
mM	Millimolar (millimoles per litre)
mm	Millimetre
MYB	Class of transcription factor
NA	Not applicable
ng	Nanogram
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
QTL	Quantitative trait locus/loci
RIL	Recombinant inbred line
RNA	Ribonucleic acid
SD	Segregation distortion
SNP	Single nucleotide polymorphism
SOE-PCR	Splicing by overlap extension-polymerase chain reaction

SS	St-0 x Sf-2
SSC	St-0 x Sf-2 Constitutive
SSD	St-0 x Sf-2 Damaged
SSR	St-0 x Sf-2 Response
TAD	Transcriptional activation domain
UTR	Untranslated region
UV-B	Ultraviolet B
WDR	WD repeat

## ABBREVIATIONS OF GENES

All genes are in *Arabidopsis thaliana* unless otherwise stated.

<i>AtMYC1</i>	Epidermal development pathway bHLH
<i>C1</i>	MYB gene regulating anthocyanin production ( <i>Zea mays</i> )
<i>CPC</i>	<i>CAPRICE</i> ; Epidermal development pathway R3 MYB repressor
<i>CRY2</i>	<i>CRYPTOCHROME 2</i> ; light-sensing protein
<i>EGL3</i>	<i>ENHANCER OF GLABRA3</i> ; Epidermal development pathway bHLH
<i>ETC1</i>	<i>ENHANCER OF TRYPTICHON AND CAPRICE 1</i> ; Epidermal development pathway R3 MYB repressor
<i>ETC2</i>	<i>ENHANCER OF TRYPTICHON AND CAPRICE 2</i> ; Epidermal development pathway R3 MYB repressor
<i>ETC3</i>	<i>ENHANCER OF TRYPTICHON AND CAPRICE 3</i> ; Epidermal development pathway R3 MYB repressor
<i>FLC</i>	<i>FLOWERING LOCUS C</i> ; MADS-box transcription factor, regulates floral transition
<i>FRI</i>	<i>FRIGIDA</i> ; flowering time determinant, confers vernalisation requirement
<i>FT</i>	<i>FLOWERING LOCUS T</i> ; promotes flowering
<i>GA1</i>	<i>GIBBERELLIC ACID REQUIRING</i> ; gibberellin biosynthetic gene
<i>GI</i>	<i>GIGANTEA</i> ; phytochrome B signalling pathway gene
<i>GL1</i>	<i>GLABRA 1</i> ; Epidermal development pathway R2R3 MYB
<i>GL2</i>	<i>GLABRA 2</i> ; Epidermal development pathway downstream target gene
<i>GL3</i>	<i>GLABRA 3</i> ; Epidermal development pathway bHLH
<i>JAZ2</i>	<i>JASMONATE-ZIM-DOMAIN PROTEIN 2</i> ; jasmonic acid response regulator
<i>JAZ9</i>	<i>JASMONATE-ZIM-DOMAIN PROTEIN 9</i> ; jasmonic acid response regulator
<i>MYB113</i>	Epidermal development pathway R2R3 MYB
<i>MYB114</i>	Epidermal development pathway R2R3 MYB
<i>MYB23</i>	Epidermal development pathway R2R3 MYB
<i>MYB5</i>	Epidermal development pathway R2R3 MYB
<i>MYBL2</i>	<i>MYB-LIKE 2</i> ; Epidermal development pathway MYB-like repressor
<i>PAP1</i>	<i>PRODUCTION OF ANTHOCYANIN 1</i> ; Epidermal development pathway R2R3 MYB

<i>PAP2</i>	<i>PRODUCTION OF ANTHOCYANIN 2</i> ; Epidermal development pathway R2R3 MYB
<i>RGL1</i>	<i>REPRESSOR OF GA-LIKE 1</i> ; DELLA-protein encoding, negative regulator of gibberellin response
<i>RPM1</i>	<i>RESISTANCE TO P. SYRINGAE PV MACULICOLA 1</i> ; Defence response
<i>SRK</i>	<i>S-Receptor Kinase</i> ; Self-incompatibility gene ( <i>Brassica</i> )
<i>TCL1</i>	<i>TRICHOMELESS 1</i> ; Epidermal development pathway R3 MYB repressor
<i>TCL2</i>	<i>TRICHOMELESS 2</i> ; Epidermal development pathway R3 MYB repressor
<i>TRY</i>	<i>TRYPTICHON</i> ; Epidermal development pathway R3 MYB repressor
<i>TT2</i>	<i>TRANSPARENT TESTA 2</i> ; Epidermal development pathway R2R3 MYB
<i>TT8</i>	<i>TRANSPARENT TESTA 8</i> ; Epidermal development pathway bHLH
<i>TTG1</i>	<i>TRANSPARENT TESTA GLABRA 1</i> ; Epidermal development pathway WDR
<i>TTG2</i>	<i>TRANSPARENT TESTA GLABRA 2</i> ; Epidermal development pathway downstream target gene
<i>URM9/SAD2</i>	<i>UNARMED9/SENSITIVE TO ABA AND DROUGHT2</i> ; importin $\beta$ -like protein linking jasmonic acid signalling to trichome initiation
<i>WDR1</i>	WDR gene regulating floral anthocyanin production ( <i>Ipomoea</i> spp)
<i>WER</i>	<i>WEREWOLF</i> ; Epidermal development pathway R2R3 MYB

